36

33

TECH UENTER 1600,2900

OCT 2 2 2001

E TRADENTE LISTING

- <110> ARNOLD, Frances H.
 PETROUNIA, Ionna P.
 SUN, Lianhong
- <120> DIRECTED EVOLUTION OF OXIDASE ENZYMES
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- <141> 2000-11-27
- <150> US 09/571,553
- <151> 2000-05-16
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Thr Cys Asp Ser Ala Gln Ser Gly Asn Glu Cys Asn Lys Ala Ile Asp
                                 25
Gly Asn Lys Asp Thr Phe Trp His Thr Phe Tyr Gly Ala Asn Gly Asp
                            40
Pro Lys Pro Pro His Thr Tyr Thr Ile Asp Met Lys Thr Thr Gln Asn
                        55
Val Asn Gly Leu Ser Met Leu Pro Arg Gln Asp Gly Asn Gln Asn Gly
Trp Ile Gly Arg His Glu Val Tyr Leu Ser Ser Asp Gly Thr Asn Trp
Gly Ser Pro Val Ala Ser Gly Ser Trp Phe Ala Asp Ser Thr Thr Lys
                                105
            100
Tyr Ser Asn Phe Glu Thr Arg Pro Ala Arg Tyr Val Arg Leu Val Ala
                                                 125
                            120
Ile Thr Glu Ala Asn Gly Gln Pro Trp Thr Ser Ile Ala Glu Ile Asn
                                             140
                        135
Val Phe Gln Ala Ser Ser Tyr Thr Ala Pro Gln Pro Gly Leu Gly Arg
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                    150
Trp Gly Pro Thr Ile Asp Leu Pro Ile Val Pro Ala Ala Ala Ile
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Glu Pro Thr Ser Gly Arg Val Leu Met Trp Ser Ser Tyr Arg Asn Asp
                                 185
            180
Ala Phe Gly Gly Ser Pro Gly Gly Ile Thr Leu Thr Ser Ser Trp Asp
        195
                            200
Pro Ser Thr Gly Ile Val Ser Asp Arg Thr Val Thr Val Thr Lys His
                                             220
                        215
Asp Met Phe Cys Pro Gly Ile Ser Met Asp Gly Asn Gly Gln Ile Val
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225 Val	Thr	Gly	Gly	Asn	230 Asp	Ala	Lys	Lys		235 Ser	Leu	Tyr	Asp	Ser	240 Ser
Ser	Asp	Ser	Trp	245 Ile	Pro	Gly	Pro		250 Met	Gln	Val	Ala	Arg	255 Gly	Tyr
Gln	Ser		260 Ala	Thr	Met	Ser		265 Gly	Arg	Val	Phe		270 Ile	Gly	Gly
Ser	Trp 290	275 Ser	Gly	Gly	Val	Phe	280 Glu	Lys	Asn	Gly		285 Val	Tyr	Ser	Pro
Ser 305		Lys	Thr	Trp	Thr 310	295 Ser	Leu	Pro	Asn	Ala 315	300 Lys	Val	Asn	Pro	Met 320
	Thr	Ala	Asp	Lys 325	Gln	Gly	Leu	Tyr	Arg 330		Asp	Asn	His	Ala 335	
Leu	Phe	Gly	Trp 340	Lys	Lys	Gly	Ser	Val 345		Gln	Ala	Gly	Pro 350		Thr
		355			Tyr		360					365	_		
	370				Asn	375					380				
385					Asp 390					395					400
				405	Gln				410					415	
			420		Pro			425					430		
		435			Ala		440					445			
	450				Ile	455					460				
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			500		Cys			505					510	_	-
		515			Tyr		520					525			
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545					550 Ser					555					560
				565	Thr				570					575	
			580		Gly			585					590		
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350

345

340

Ala Met Asn Trp Tyr Tyr Thr Ser Gly Ser Gly Asp Val Lys Ser Ala 360 Gly Lys Arg Gln Ser Asn Arg Gly Val Ala Pro Asp Ala Met Cys Gly 375 380 Asn Ala Val Met Tyr Asp Ala Val Lys Gly Lys Ile Leu Thr Phe Gly 390 395 Gly Ser Pro Asp Tyr Gln Asp Ser Asp Ala Thr Thr Asn Ala His Ile 405 410 Ile Thr Leu Gly Glu Pro Gly Thr Ser Pro Asn Thr Val Phe Ala Ser 425 Asn Gly Leu Tyr Phe Ala Arg Thr Phe His Thr Ser Val Val Leu Pro 435 440445 Asp Gly Ser Thr Phe Ile Thr Gly Gly Gln Arg Arg Gly Ile Pro Phe 455 Glu Asp Ser Thr Pro Val Phe Thr Pro Glu Ile Tyr Val Pro Glu Gln 470 475 Asp Thr Phe Tyr Lys Gln Asn Pro Asn Ser Ile Val Arg Ala Tyr His 490 485 Ser Ile Ser Leu Leu Leu Pro Asp Gly Arg Val Phe Asn Gly Gly 505 Gly Leu Ser Gly Asp Cys Thr Thr Asn His Phe Asp Ala Gln Ile Phe 520 515 Thr Pro Asn Tyr Leu Tyr Asn Ser Asn Gly Asn Leu Ala Thr Arg Pro 535 Lys Ile Thr Arg Thr Ser Thr Gln Ser Val Lys Val Gly Gly Arg Ile 550 555 Thr Ile Ser Thr Asp Ser Ser Ile Ser Lys Ala Ser Leu Ile Arg Tyr 565 570 Gly Thr Ala Thr His Thr Val Asn Thr Asp Gln Arg Arg Ile Pro Leu 580 585 Thr Leu Thr Asn Asn Gly Gly Asn Ser Tyr Ser Phe Gln Val Pro Ser 600 605 Asp Ser Gly Val Ala Leu Pro Gly Tyr Trp Met Leu Phe Val Met Asn 615 Ser Ala Gly Val Pro Ser Val Ala Ser Thr Ile Arg Val Thr Gln <210> 12 <211> 639 <212> PRT <213> Artificial Sequence <220> <223> Mutant 9.16.16D12 (P136, V494A) of D. dendroides GaO <400> 12 Ala Ser Ala Pro Ile Gly Ser Ala Ile Ser Arg Asn Asn Trp Ala Val 10 Thr Cys Asp Ser Ala Gln Ser Gly Asn Glu Cys Asn Lys Ala Ile Asp

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Gly Asn Lys Asp Thr Phe Trp His Thr Phe Tyr Gly Ala Asn Gly Asp

40

Pro Lys Pro Pro His Thr Tyr Thr Ile Asp Met Lys Thr Thr Gln Asn Val Asn Gly Leu Ser Met Leu Pro Arg Gln Asp Gly Asn Gln Asn Gly Trp Ile Gly Arg His Glu Val Tyr Leu Ser Ser Asp Gly Thr Asn Trp Gly Ser Pro Val Ala Ser Gly Ser Trp Phe Ala Asp Ser Thr Thr Lys Tyr Ser Asn Phe Glu Thr Arg Pro Ala Arg Tyr Val Arg Leu Val Ala Ile Thr Glu Ala Asn Gly Gln Pro Trp Thr Ser Ile Ala Glu Ile Asn Val Phe Gln Ala Ser Ser Tyr Thr Ala Pro Gln Pro Gly Leu Gly Arg Trp Gly Pro Thr Ile Asp Leu Pro Ile Val Pro Ala Ala Ala Ile Glu Pro Thr Ser Gly Arg Val Leu Met Trp Ser Ser Tyr Arg Asn Asp Ala Phe Gly Gly Ser Pro Gly Gly Ile Thr Leu Thr Ser Ser Trp Asp Pro Ser Thr Gly Ile Val Ser Asp Arg Thr Val Thr Val Thr Lys His Asp Met Phe Cys Pro Gly Ile Ser Met Asp Gly Asn Gly Gln Ile Val Val Thr Gly Gly Asn Asp Ala Lys Lys Thr Ser Leu Tyr Asp Ser Ser Ser Asp Ser Trp Ile Pro Gly Pro Asp Met Gln Val Ala Arg Gly Tyr Gln Ser Ser Ala Thr Met Ser Asp Gly Arg Val Phe Thr Ile Gly Gly Ser Trp Ser Gly Gly Val Phe Glu Lys Asn Gly Glu Val Tyr Ser Pro Ser Ser Lys Thr Trp Thr Ser Leu Pro Asn Ala Lys Val Asn Pro Met Leu Thr Ala Asp Lys Gln Gly Leu Tyr Arg Ser Asp Asn His Ala Trp Leu Phe Gly Trp Lys Lys Gly Ser Val Phe Gln Ala Gly Pro Ser Thr Ala Met Asn Trp Tyr Tyr Thr Ser Gly Ser Gly Asp Val Lys Ser Ala Gly Lys Arq Gln Ser Asn Arg Gly Val Ala Pro Asp Ala Met Cys Gly Asn Ala Val Met Tyr Asp Ala Val Lys Gly Lys Ile Leu Thr Phe Gly Gly Ser Pro Asp Tyr Gln Asp Ser Asp Ala Thr Thr Asn Ala His Ile Ile Thr Leu Gly Glu Pro Gly Thr Ser Pro Asn Thr Val Phe Ala Ser Asn Gly Leu Tyr Phe Ala Arg Thr Phe His Thr Ser Val Val Leu Pro Asp Gly Ser Thr Phe Ile Thr Gly Gly Gln Arg Arg Gly Ile Pro Phe Glu Asp Ser Thr Pro Val Phe Thr Pro Glu Ile Tyr Val Pro Glu Gln Page 7

475 470 465 Asp Thr Phe Tyr Lys Gln Asn Pro Asn Ser Ile Val Arg Ala Tyr His 490 485 Ser Ile Ser Leu Leu Pro Asp Gly Arg Val Phe Asn Gly Gly 505 500 Gly Leu Cys Gly Asp Cys Thr Thr Asn His Phe Asp Ala Gln Ile Phe 520 525 Thr Pro Asn Tyr Leu Tyr Asn Ser Asn Gly Asn Leu Ala Thr Arg Pro 535 540 Lys Ile Thr Arg Thr Ser Thr Gln Ser Val Lys Val Gly Gly Arg Ile 555 550 Thr Ile Ser Thr Asp Ser Ser Ile Ser Lys Ala Ser Leu Ile Arg Tyr 565 570 Gly Thr Ala Thr His Thr Val Asn Thr Asp Gln Arg Arg Ile Pro Leu 585 580 Thr Leu Thr Asn Asn Gly Gly Asn Ser Tyr Ser Phe Gln Val Pro Ser 600 Asp Ser Gly Val Ala Leu Pro Gly Tyr Trp Met Leu Phe Val Met Asn 620 615 Ser Ala Gly Val Pro Ser Val Ala Ser Thr Ile Arg Val Thr Gln 630 635 625

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_				165					170	_	_	_	_	175	_
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Pro	Ser 210	Thr	Gly	Ile	Val	Ser 215	Asp	Arg	Thr	Val	Thr 220	Val	Thr	Lys	His
Asp 225		Phe	Cys	Pro	Gly 230		Ser	Met	Asp	Gly 235		Gly	Gln	Ile	Val 240
	Thr	Gly	Gly	Asn 245		Ala	Lys	Lys	Thr 250		Leu	Tyr	Asp	Ser 255	Ser
Ser	Asp	Ser	Trp 260		Pro	Gly	Pro	Asp 265		Gln	Val	Ala	Arg 270		Tyr
Gln	Ser	Ser 275		Thr	Met	Ser	Asp 280		Arg	Val	Phe	Thr 285	Ile	Gly	Gly
Ser	Trp 290		Gly	Gly	Val	Phe 295		Lys	Asn	Gly	Glu 300		Tyr	Ser	Pro
Ser 305		Lys	Thr	Trp	Thr 310		Leu	Pro	Asn	Ala 315	Lys	Val	Asn	Pro	Met 320
	Thr	Ala	Asp	Lys 325		Gly	Leu	Tyr	Arg 330	Ser	Asp	Asn	His	Ala 335	Trp
Leu	Phe	Gly	Trp 340		Lys	Gly	Ser	Val 345	Phe	Gln	Ala	Gly	Pro 350	Ser	Thr
Ala	Met	Asn 355		Tyr	Tyr	Thr	Ser 360		Ser	Gly	Asp	Val 365	Lys	Ser	Ala
Gly	Lys 370		Gln	Ser	Asn	Arg		Val	Ala	Pro	Asp 380	Ala	Met	Cys	Gly
Asn 385		Val	Met	Tyr	Asp 390	Ala	Val	Lys	Gly	Lys 395	Ile	Leu	Thr	Phe	Gly 400
	Ser	Pro	Asp	Tyr 405		Asp	Ser	Asp	Ala 410	Thr	Thr	Asn	Ala	His 415	Ile
Ile	Thr	Leu	Gly 420	Glu	Pro	Gly	Thr	Ser 425	Pro	Asn	Thr	Val	Phe 430	Ala	Ser
Asn	Gly	Leu 435	Tyr	Phe	Ala	Arg	Thr 440	Phe	His	Thr	Ser	Val 445	Val	Leu	Pro
Asp	Gly 450	Ser	Thr	Phe		Thr 455		Gly	Gln		Arg 460		Ile	Pro	Phe
Glu 465	Asp	Ser	Thr	Pro	Val 470	Phe	Thr	Pro	Glu	Ile 475	Tyr	Val	Pro	Glu	Gln 480
	Thr	Phe	Tyr	Lys 485	Gln	Asn	Pro	Asn	Ser 490	Ile	Val	Arg	Ala	Tyr 495	His
Ser	Ile	Ser	Leu 500	Leu	Leu	Pro	Asp	Gly 505	Arg	Val	Phe	Asn	Gly 510	Gly	Gly
Gly	Leu	Cys 515	Gly	Asp	Cys	Thr	Thr 520	Asn	His	Phe	Asp	Ala 525	Gln	Ile	Phe
Thr	Pro 530	Asn	Tyr	Leu	Tyr	Asn 535	Ser	Asn	Gly	Asn	Leu 540	Ala	Thr	Arg	Pro
Lys 545	Ile	Thr	Arg	Thr	Ser 550	Thr	Gln	Ser	Val	Lys 555	Val	Gly	Gly	Arg	Ile 560
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Gly	Thr	Ala	Thr 580	His	Thr	Val	Asn	585	Asp ge 9		Arg	Arg	Ile 590	Pro	Leu

Thr Leu Thr Asn Asn Gly Gly Asn Ser Tyr Ser Phe Gln Val Pro Ser Asp Ser Gly Val Ala Leu Pro Gly Tyr Trp Met Leu Phe Val Met Asn 615 620 Ser Ala Gly Val Pro Ser Val Ala Ser Thr Ile Arg Val Thr Gln 630 635 625 <210> 14 <211> 639 <212> PRT <213> Artificial Sequence <220> <223> Mutant 11.03.10C3 (A3, P136, G195E, V494A) of D. dendroides GaO <400> 14 Ala Ser Ala Pro Ile Gly Ser Ala Ile Ser Arg Asn Asn Trp Ala Val 10 Thr Cys Asp Ser Ala Gln Ser Gly Asn Glu Cys Asn Lys Ala Ile Asp Gly Asn Lys Asp Thr Phe Trp His Thr Phe Tyr Gly Ala Asn Gly Asp 40 Pro Lys Pro Pro His Thr Tyr Thr Ile Asp Met Lys Thr Thr Gln Asn 55 Val Asn Gly Leu Ser Met Leu Pro Arg Gln Asp Gly Asn Gln Asn Gly 75 Trp Ile Gly Arg His Glu Val Tyr Leu Ser Ser Asp Gly Thr Asn Trp 90 Gly Ser Pro Val Ala Ser Gly Ser Trp Phe Ala Asp Ser Thr Thr Lys 105 Tyr Ser Asn Phe Glu Thr Arg Pro Ala Arg Tyr Val Arg Leu Val Ala 120 125 Ile Thr Glu Ala Asn Gly Gln Pro Trp Thr Ser Ile Ala Glu Ile Asn 135 140 Val Phe Gln Ala Ser Ser Tyr Thr Ala Pro Gln Pro Gly Leu Gly Arg 155 150 Trp Gly Pro Thr Ile Asp Leu Pro Ile Val Pro Ala Ala Ala Ile 165 170 175 Glu Pro Thr Ser Gly Arg Val Leu Met Trp Ser Ser Tyr Arg Asn Asp Ala Phe Glu Gly Ser Pro Gly Gly Ile Thr Leu Thr Ser Ser Trp Asp 200 205 Pro Ser Thr Gly Ile Val Ser Asp Arg Thr Val Thr Val Thr Lys His 220 215 Asp Met Phe Cys Pro Gly Ile Ser Met Asp Gly Asn Gly Gln Ile Val 235 230 Val Thr Gly Gly Asn Asp Ala Lys Lys Thr Ser Leu Tyr Asp Ser Ser 250 245 Ser Asp Ser Trp Ile Pro Gly Pro Asp Met Gln Val Ala Arg Gly Tyr 265 260 Gln Ser Ser Ala Thr Met Ser Asp Gly Arg Val Phe Thr Ile Gly Gly

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Ser Trp Ser Gly Gly Val Phe Glu Lys Asn Gly Glu Val Tyr Ser Pro 295 Ser Ser Lys Thr Trp Thr Ser Leu Pro Asn Ala Lys Val Asn Pro Met 315 310 Leu Thr Ala Asp Lys Gln Gly Leu Tyr Arg Ser Asp Asn His Ala Trp 325 330 Leu Phe Gly Trp Lys Lys Gly Ser Val Phe Gln Ala Gly Pro Ser Thr 345 Ala Met Asn Trp Tyr Tyr Thr Ser Gly Ser Gly Asp Val Lys Ser Ala 355 360 365 Gly Lys Arg Gln Ser Asn Arg Gly Val Ala Pro Asp Ala Met Cys Gly 375 Asn Ala Val Met Tyr Asp Ala Val Lys Gly Lys Ile Leu Thr Phe Gly 390 395 Gly Ser Pro Asp Tyr Gln Asp Ser Asp Ala Thr Thr Asn Ala His Ile 405 410 Ile Thr Leu Gly Glu Pro Gly Thr Ser Pro Asn Thr Val Phe Ala Ser 425 430 420 Asn Gly Leu Tyr Phe Ala Arg Thr Phe His Thr Ser Val Val Leu Pro 440 445 Asp Gly Ser Thr Phe Ile Thr Gly Gly Gln Arg Arg Gly Ile Pro Phe 450 455 Glu Asp Ser Thr Pro Val Phe Thr Pro Glu Ile Tyr Val Pro Glu Gln 475 470 Asp Thr Phe Tyr Lys Gln Asn Pro Asn Ser Ile Val Arg Ala Tyr His 495 485 490 Ser Ile Ser Leu Leu Pro Asp Gly Arg Val Phe Asn Gly Gly 505 Gly Leu Cys Gly Asp Cys Thr Thr Asn His Phe Asp Ala Gln Ile Phe 520 Thr Pro Asn Tyr Leu Tyr Asn Ser Asn Gly Asn Leu Ala Thr Arg Pro 535 540 Lys Ile Thr Arg Thr Ser Thr Gln Ser Val Lys Val Gly Gly Arg Ile 550 555 560 Thr Ile Ser Thr Asp Ser Ser Ile Ser Lys Ala Ser Leu Ile Arg Tyr 570 565 Gly Thr Ala Thr His Thr Val Asn Thr Asp Gln Arg Arg Ile Pro Leu 585 580 Thr Leu Thr Asn Asn Gly Gly Asn Ser Tyr Ser Phe Gln Val Pro Ser 600 Asp Ser Gly Val Ala Leu Pro Gly Tyr Trp Met Leu Phe Val Met Asn 615 620 Ser Ala Gly Val Pro Ser Val Ala Ser Thr Ile Arg Val Thr Gln 635 625 630

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405 410 Ile Thr Leu Gly Glu Pro Gly Thr Ser Pro Asn Thr Val Phe Ala Ser 420 425 Asn Gly Leu Tyr Phe Ala Arg Thr Phe His Thr Ser Val Val Leu Pro 440 435 445 Asp Gly Ser Thr Phe Ile Thr Gly Gly Gln Arg Arg Gly Ile Pro Phe 455 Glu Asp Ser Thr Pro Val Phe Thr Pro Glu Ile Tyr Val Pro Glu Gln 470 475 Asp Thr Phe Tyr Lys Gln Asn Pro Asn Ser Ile Val Arg Ala Tyr His 485 490 Ser Ile Ser Leu Leu Pro Asp Gly Arg Val Phe Asn Gly Gly Gly 505 Gly Leu Cys Gly Asp Cys Thr Thr Asn His Phe Asp Ala Gln Ile Phe 515 520 525 Thr Pro Asn Tyr Leu Tyr Asp Ser Asn Gly Asn Leu Ala Thr Arg Pro 535 540 Lys Ile Thr Arg Thr Ser Thr Gln Ser Val Lys Val Gly Gly Arg Ile 550 555 Thr Ile Ser Thr Asp Ser Ser Ile Ser Lys Ala Ser Leu Ile Arg Tyr 565 570 Gly Thr Ala Thr His Thr Val Asn Thr Asp Gln Arg Arg Ile Pro Leu 580 585 Thr Leu Thr Asn Asn Gly Gly Asn Ser Tyr Ser Phe Gln Val Pro Ser 600 605 Asp Ser Gly Val Ala Leu Pro Gly Tyr Trp Met Leu Phe Val Met Asn 615 Ser Ala Gly Val Pro Ser Val Ala Ser Thr Ile Arg Val Thr Gln

<210> 16

<211> 639

<212> PRT

<213> Artificial Sequence

<220>

<223> Mutant 11.03.13E12 (M70V, P136, V494A) from D. Dendroides GaO

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Val 145	Phe	Gln	Ala	Ser	Ser 150	Tyr	Thr	Ala	Pro	Gln 155	Pro	Gly	Leu	Gly	Arg 160
Trp	Gly	Pro	Thr	Ile 165	Asp	Leu	Pro	Ile	Val 170	Pro	Ala	Ala	Ala	Ala 175	Ile
Glu	Pro	Thr	Ser 180	Gly	Arg	Val	Leu	Met 185	Trp	Ser	Ser	Tyr	Arg 190	Asn	Asp
Ala	Phe	Gly 195	Gly	Ser	Pro	Gly	Gly 200	Ile	Thr	Leu	Thr	Ser 205	Ser	Trp	Asp
Pro	Ser 210	Thr	Gly	Ile	Val	Ser 215	Asp	Arg	Thr	Val	Thr 220	Val	Thr	Lys	His
Asp 225	Met	Phe	Cys	Pro	Gly 230	Ile	Ser	Met	Asp	Gly 235	Asn	Gly	Gln	Ile	Val 240
			_	245		Ala			250					255	
Ser	Asp	Ser	Trp 260	Ile	Pro	Gly	Pro	Asp 265	Met	Gln	Val	Ala	Arg 270	Gly	Tyr
Gln	Ser	Ser 275	Ala	Thr	Met	Ser	Asp 280	Gly	Arg	Val	Phe	Thr 285	Ile	Gly	Gly
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305					310	Ser				315					320
			_	325		Gly		_	330					335	
			340			Gly		345					350		
		355	_	_		Thr	360					365			
_	370	_				Arg 375	_				380				
385				_	390	Ala			_	395					400
_			_	405					410					415	Ile
			420			_		425					430		Ser
	-	435	_			Arg	440					445			
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465	_				470					475	_				Gln 480
_			_	485		Asn			490					495	
			500					505					510		Gly
Gly	Leu	Cys 515	Gly	Asp	Cys	Thr	Thr 520				Asp	Ala 525	Gln	11e	Pne
								Pac	70 14	4					

Thr Pro Asn Tyr Leu Tyr Asn Ser Asn Gly Asn Leu Ala Thr Arg Pro 535 Lys Ile Thr Arg Thr Ser Thr Gln Ser Val Lys Val Gly Gly Arg Ile 550 555 Thr Ile Ser Thr Asp Ser Ser Ile Ser Lys Ala Ser Leu Ile Arg Tyr 565 570 Gly Thr Ala Thr His Thr Val Asn Thr Asp Gln Arg Arg Ile Pro Leu 585 Thr Leu Thr Asn Asn Gly Gly Asn Ser Tyr Ser Phe Gln Val Pro Ser 600 Asp Ser Gly Val Ala Leu Pro Gly Tyr Trp Met Leu Phe Val Met Asn 615 620 Ser Ala Gly Val Pro Ser Val Ala Ser Thr Ile Arg Val Thr Gln 630 635 625 <210> 17 <211> 639 <212> PRT <213> Artificial Sequence <220> <223> Mutant 1.06.20E7 (S10P, M70V, P136, G195E, V494A, N535D) from D. Dendroides GaO <400> 17 Ala Ser Ala Pro Ile Gly Ser Ala Ile Pro Arg Asn Asn Trp Ala Val Thr Cys Asp Ser Ala Gln Ser Gly Asn Glu Cys Asn Lys Ala Ile Asp 25 Gly Asn Lys Asp Thr Phe Trp His Thr Phe Tyr Gly Ala Asn Gly Asp 40 Pro Lys Pro Pro His Thr Tyr Thr Ile Asp Met Lys Thr Thr Gln Asn 55 Val Asn Gly Leu Ser Val Leu Pro Arg Gln Asp Gly Asn Gln Asn Gly 70 Trp Ile Gly Arg His Glu Val Tyr Leu Ser Ser Asp Gly Thr Asn Trp 90 Gly Ser Pro Val Ala Ser Gly Ser Trp Phe Ala Asp Ser Thr Thr Lys 105 110 Tyr Ser Asn Phe Glu Thr Arg Pro Ala Arg Tyr Val Arg Leu Val Ala 115 120 Ile Thr Glu Ala Asn Gly Gln Pro Trp Thr Ser Ile Ala Glu Ile Asn 135 140 Val Phe Gln Ala Ser Ser Tyr Thr Ala Pro Gln Pro Gly Leu Gly Arg 150 155 Trp Gly Pro Thr Ile Asp Leu Pro Ile Val Pro Ala Ala Ala Ile 170 Glu Pro Thr Ser Gly Arg Val Leu Met Trp Ser Ser Tyr Arg Asn Asp 180 185 Ala Phe Glu Gly Ser Pro Gly Gly Ile Thr Leu Thr Ser Ser Trp Asp 200 195 Pro Ser Thr Gly Ile Val Ser Asp Arg Thr Val Thr Val Thr Lys His 210 215

Asp Met Phe Cys Pro Gly Ile Ser Met Asp Gly Asn Gly Gln Ile Val Val Thr Gly Gly Asn Asp Ala Lys Lys Thr Ser Leu Tyr Asp Ser Ser Ser Asp Ser Trp Ile Pro Gly Pro Asp Met Gln Val Ala Arg Gly Tyr Gln Ser Ser Ala Thr Met Ser Asp Gly Arg Val Phe Thr Ile Gly Gly Ser Trp Ser Gly Gly Val Phe Glu Lys Asn Gly Glu Val Tyr Ser Pro Ser Ser Lys Thr Trp Thr Ser Leu Pro Asn Ala Lys Val Asn Pro Met Leu Thr Ala Asp Lys Gln Gly Leu Tyr Arg Ser Asp Asn His Ala Trp Leu Phe Gly Trp Lys Lys Gly Ser Val Phe Gln Ala Gly Pro Ser Thr Ala Met Asn Trp Tyr Tyr Thr Ser Gly Ser Gly Asp Val Lys Ser Ala Gly Lys Arg Gln Ser Asn Arg Gly Val Ala Pro Asp Ala Met Cys Gly Asn Ala Val Met Tyr Asp Ala Val Lys Gly Lys Ile Leu Thr Phe Gly Gly Ser Pro Asp Tyr Gln Asp Ser Asp Ala Thr Thr Asn Ala His Ile Ile Thr Leu Gly Glu Pro Gly Thr Ser Pro Asn Thr Val Phe Ala Ser Asn Gly Leu Tyr Phe Ala Arg Thr Phe His Thr Ser Val Val Leu Pro Asp Gly Ser Thr Phe Ile Thr Gly Gly Gln Arg Arg Gly Ile Pro Phe Glu Asp Ser Thr Pro Val Phe Thr Pro Glu Ile Tyr Val Pro Glu Gln Asp Thr Phe Tyr Lys Gln Asn Pro Asn Ser Ile Val Arg Ala Tyr His Ser Ile Ser Leu Leu Pro Asp Gly Arg Val Phe Asn Gly Gly Gly Leu Cys Gly Asp Cys Thr Thr Asn His Phe Asp Ala Gln Ile Phe Thr Pro Asn Tyr Leu Tyr Asp Ser Asn Gly Asn Leu Ala Thr Arg Pro Lys Ile Thr Arg Thr Ser Thr Gln Ser Val Lys Val Gly Gly Arg Ile Thr Ile Ser Thr Asp Ser Ser Ile Ser Lys Ala Ser Leu Ile Arg Tyr Gly Thr Ala Thr His Thr Val Asn Thr Asp Gln Arg Arg Ile Pro Leu Thr Leu Thr Asn Asn Gly Gly Asn Ser Tyr Ser Phe Gln Val Pro Ser Asp Ser Gly Val Ala Leu Pro Gly Tyr Trp Met Leu Phe Val Met Asn Ser Ala Gly Val Pro Ser Val Ala Ser Thr Ile Arg Val Thr Gln

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Ala Met Asn Trp Tyr Tyr Thr Ser Gly Ser Gly Asp Val Lys Ser Ala 360 Gly Lys Arg Gln Ser Asn Arg Gly Val Ala Pro Asp Ala Met Cys Gly 375 380 Asn Ala Val Met Tyr Asp Ala Val Lys Gly Lys Ile Leu Thr Phe Gly 390 395 Gly Ser Pro Asp Tyr Gln Asp Ser Asp Ala Thr Thr Asp Ala His Ile 410 Ile Thr Leu Gly Glu Pro Gly Thr Ser Pro Asn Thr Val Phe Ala Ser 420 425 430 Asn Gly Leu Tyr Phe Ala Arg Thr Phe His Thr Ser Val Val Leu Pro 440 Asp Gly Ser Thr Phe Ile Thr Gly Gly Gln Arg Arg Gly Ile Pro Phe 455 Glu Asp Ser Thr Pro Val Phe Thr Pro Glu Ile Tyr Val Pro Glu Gln 470 475 Asp Thr Phe Tyr Lys Gln Asn Pro Asn Ser Ile Val Arg Val Tyr His 485 490 Ser Ile Ser Leu Leu Pro Asp Gly Arg Val Phe Asn Gly Gly 500 505 Gly Leu Cys Gly Asp Cys Thr Thr Asn His Phe Asp Ala Gln Ile Phe 515 520 Thr Pro Asn Tyr Leu Tyr Asn Ser Asn Gly Asn Leu Ala Thr Arg Pro 535 540 Lys Ile Thr Arg Thr Ser Thr Gln Ser Val Lys Val Gly Gly Arg Ile 550 555 Thr Ile Ser Thr Asp Ser Ser Ile Ser Lys Ala Ser Leu Ile Arg Tyr 565 570 Gly Thr Ala Thr His Thr Val Asn Thr Asp Gln Arg Arg Ile Pro Leu 580 585 Thr Leu Thr Asn Asn Gly Gly Asn Ser Tyr Ser Phe Gln Val Pro Ser 600 Asp Ser Gly Val Ala Leu Pro Gly Tyr Trp Met Leu Phe Val Met Asn 615 620 Ser Ala Gly Val Pro Ser Val Ala Ser Thr Ile Arg Val Thr Gln 625 630 <210> 19 <211> 639 <212> PRT <213> Artificial Sequence <220> <223> Mutant 2.G4 (N413D, S550) from D. Dendroides GaO <400> 19 Ala Ser Ala Pro Ile Gly Ser Ala Ile Ser Arg Asn Asn Trp Ala Val Thr Cys Asp Ser Ala Gln Ser Gly Asn Glu Cys Asn Lys Ala Ile Asp 25 Gly Asn Lys Asp Thr Phe Trp His Thr Phe Tyr Gly Ala Asn Gly Asp 40 Pro Lys Pro Pro His Thr Tyr Thr Ile Asp Met Lys Thr Thr Gln Asn

	50 55 60														
Val 65	Asn	Gly	Leu	Ser	Met 70	Leu	Pro	Arg	Gln	Asp 75	Gly	Asn	Gln	Asn	Gly 80
Trp	Ile	Gly	Arg	His 85	Glu	Val	Tyr	Leu	Ser 90	Ser	Asp	Gly	Thr	Asn 95	Trp
Gly	Ser	Pro	Val 100	Ala	Ser	Gly	Ser	Trp 105	Phe	Ala	Asp	Ser	Thr 110		Lys
Tyr	Ser	Asn 115	Phe	Glu	Thr	Arg	Pro 120		Arg	Tyr	Val	Arg 125		Val	Ala
Ile	Thr 130	Glu	Ala	Asn	Gly	Gln 135	Pro	Trp	Thr	Ser	Ile 140		Glu	Ile	Asn
Val 145	Phe	Gln	Ala	Ser	Ser 150	Tyr	Thr	Ala	Pro	Gln 155	Pro	Gly	Leu	Gly	Arg 160
Trp	Gly	Pro	Thr	Ile 165	Asp	Leu	Pro	Ile	Val 170		Ala	Ala	Ala	Ala 175	
Glu	Pro	Thr	Ser 180	Gly	Arg	Val	Leu	Met 185	Trp	Ser	Ser	Tyr	Arg		Asp
Ala	Phe	Gly 195	Gly	Ser	Pro	Gly	Gly 200	Ile	Thr	Leu	Thr	Ser 205	Ser	Trp	Asp
	210				Val	215					220				
225					Gly 230					235		_			240
				245	Asp				250				_	255	
			260		Pro			265					270		_
		275			Met		280					285			_
	290				Val	295		_		_	300		-		
305					Thr 310					315					320
				325	Gln				330		_			335	-
			340		Lys			345					350		
		355			Tyr		360					365			
	370				Asn	375					380			_	_
385					Asp 390					395					400
				405	Gln				410					415	
			420		Pro			425					430		
		435			Ala		440					445			
	450				Ile	455					460				
G1u 465	Asp	ser	Inr	Pro	Val 470	Phe	Thr			475	Tyr	Val	Pro	Glu	Gln 480
								Pag	je 19)					

Asp Thr Phe Tyr Lys Gln Asn Pro Asn Ser Ile Val Arg Val Tyr His 485 Ser Ile Ser Leu Leu Pro Asp Gly Arg Val Phe Asn Gly Gly Gly 505 Gly Leu Cys Gly Asp Cys Thr Thr Asn His Phe Asp Ala Gln Ile Phe 515 520 525 Thr Pro Asn Tyr Leu Tyr Asn Ser Asn Gly Asn Leu Ala Thr Arg Pro 535 Lys Ile Thr Arg Thr Ser Thr Gln Ser Val Lys Val Gly Gly Arg Ile 550 555 560 Thr Ile Ser Thr Asp Ser Ser Ile Ser Lys Ala Ser Leu Ile Arg Tyr 570 565 Gly Thr Ala Thr His Thr Val Asn Thr Asp Gln Arg Arg Ile Pro Leu 585 Thr Leu Thr Asn Asn Gly Gly Asn Ser Tyr Ser Phe Gln Val Pro Ser 600 605 Asp Ser Gly Val Ala Leu Pro Gly Tyr Trp Met Leu Phe Val Met Asn 615 620 Ser Ala Gly Val Pro Ser Val Ala Ser Thr Ile Arg Val Thr Gln 625 630 635

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<212> PRT

<213> Artificial Sequence

<220>

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Glu	Pro	Thr	Ser 180	Gly	Arg	Val	Leu	Met 185	Trp	Ser	Ser	Tyr	Arg 190	Asn	Asp
Ala	Phe	Gly 195		Ser	Pro	Gly	Gly 200		Thr	Leu	Thr	Ser 205	Ser	Trp	Asp
Pro	Ser 210	Thr	Gly	Ile	Val	Ser 215	Asp	Arg	Thr	Val	Thr 220	Val	Thr	Lys	His
Asp 225	Met	Phe	Cys	Pro	Gly 230	Ile	Ser	Met	Asp	Gly 235	Asn	Gly	Gln	Ile	Val 240
Val	Thr	Gly	Gly	Asn 245	Asp	Ala	Lys	Lys	Thr 250	Ser	Leu	Tyr	Asp	Ser 255	Ser
	_		260			_	Pro	265					270	_	_
		275					Asp 280	-				285			
	290					295	Glu				300		_		
305		_		_	310		Leu			315	_				320
				325			Leu		330					335	
		_	340	_	_	_	Ser	345				_	350		
		355					Ser 360					365			
_	370					375	Gly				380			_	_
385				_	390		Val	_	_	395					400
			_	405		_	Ser	_	410			_		415	
			420				Thr	425					430		
		435					Thr					445			
_	450^{-}					455	Gly Thr	-		_	460	-			
465					470					475					480
			_	485			Pro		490					495	
			500				Asp	505	_				510		
		515	_		_		Thr 520				_	525			
	530		_		_	535	Ser		_		540			_	
545			_		550		Gln			555		_	_	_	560
				565			Ile		570					575	_
			580				Asn	585					590		
TIIL	пеп	1111.	ASII	ASII	дтλ	σтλ	Asn		ge 21		rne	GIII	val	PLO	ser

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600 605 Asp Ser Gly Val Ala Leu Pro Gly Tyr Trp Met Leu Phe Val Met Asn 615 620 Ser Ala Gly Val Pro Ser Val Ala Ser Thr Ile Arg Val Thr Gln 630 <210> 21 <211> 639 <212> PRT <213> Artificial Sequence <220> <223> Mutant 4.F12 (N413D, S550, V494A, S610) from D. Dendroides GaO <400> 21 Ala Ser Ala Pro Ile Gly Ser Ala Ile Ser Arg Asn Asn Trp Ala Val Thr Cys Asp Ser Ala Gln Ser Gly Asn Glu Cys Asn Lys Ala Ile Asp 25 Gly Asn Lys Asp Thr Phe Trp His Thr Phe Tyr Gly Ala Asn Gly Asp Pro Lys Pro Pro His Thr Tyr Thr Ile Asp Met Lys Thr Thr Gln Asn 55 Val Asn Gly Leu Ser Met Leu Pro Arg Gln Asp Gly Asn Gln Asn Gly 70 Trp Ile Gly Arg His Glu Val Tyr Leu Ser Ser Asp Gly Thr Asn Trp 90 Gly Ser Pro Val Ala Ser Gly Ser Trp Phe Ala Asp Ser Thr Thr Lys 100 105 Tyr Ser Asn Phe Glu Thr Arg Pro Ala Arg Tyr Val Arg Leu Val Ala 120 Ile Thr Glu Ala Asn Gly Gln Pro Trp Thr Ser Ile Ala Glu Ile Asn 135 140 Val Phe Gln Ala Ser Ser Tyr Thr Ala Pro Gln Pro Gly Leu Gly Arg 155 Trp Gly Pro Thr Ile Asp Leu Pro Ile Val Pro Ala Ala Ala Ile 170 Glu Pro Thr Ser Gly Arg Val Leu Met Trp Ser Ser Tyr Arg Asn Asp 180 185 Ala Phe Gly Gly Ser Pro Gly Gly Ile Thr Leu Thr Ser Ser Trp Asp 200 Pro Ser Thr Gly Ile Val Ser Asp Arg Thr Val Thr Lys His 215 220 Asp Met Phe Cys Pro Gly Ile Ser Met Asp Gly Asn Gly Gln Ile Val 230 235 Val Thr Gly Gly Asn Asp Ala Lys Lys Thr Ser Leu Tyr Asp Ser Ser 250 Ser Asp Ser Trp Ile Pro Gly Pro Asp Met Gln Val Ala Arg Gly Tyr 260 265 Gln Ser Ser Ala Thr Met Ser Asp Gly Arg Val Phe Thr Ile Gly Gly 280 Ser Trp Ser Gly Gly Val Phe Glu Lys Asn Gly Glu Val Tyr Ser Pro

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                                             300
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Leu Thr Ala Asp Lys Gln Gly Leu Tyr Arg Ser Asp Asn His Ala Trp
                                    330
Leu Phe Gly Trp Lys Lys Gly Ser Val Phe Gln Ala Gly Pro Ser Thr
            340
                                345
Ala Met Asn Trp Tyr Tyr Thr Ser Gly Ser Gly Asp Val Lys Ser Ala
                            360
Gly Lys Arg Gln Ser Asn Arg Gly Val Ala Pro Asp Ala Met Cys Gly
                        375
                                             380
Asn Ala Val Met Tyr Asp Ala Val Lys Gly Lys Ile Leu Thr Phe Gly
                    390
                                         395
Gly Ser Pro Asp Tyr Gln Asp Ser Asp Ala Thr Thr Asp Ala His Ile
                405
                                    410
Ile Thr Leu Gly Glu Pro Gly Thr Ser Pro Asn Thr Val Phe Ala Ser
            420
                                425
                                                     430
Asn Gly Leu Tyr Phe Ala Arg Thr Phe His Thr Ser Val Val Leu Pro
                            440
        435
Asp Gly Ser Thr Phe Ile Thr Gly Gly Gln Arg Arg Gly Ile Pro Phe
                        455
Glu Asp Ser Thr Pro Val Phe Thr Pro Glu Ile Tyr Val Pro Glu Gln
                    470
                                         475
Asp Thr Phe Tyr Lys Gln Asn Pro Asn Ser Ile Val Arg Ala Tyr His
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                                    490
Ser Ile Ser Leu Leu Pro Asp Gly Arg Val Phe Asn Gly Gly Gly
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                                505
Gly Leu Cys Gly Asp Cys Thr Thr Asn His Phe Asp Ala Gln Ile Phe
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                            520
                                                 525
Thr Pro Asn Tyr Leu Tyr Asn Ser Asn Gly Asn Leu Ala Thr Arg Pro
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                                             540
Lys Ile Thr Arg Thr Ser Thr Gln Ser Val Lys Val Gly Gly Arg Ile
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                                         555
                                                             560
Thr Ile Ser Thr Asp Ser Ser Ile Ser Lys Ala Ser Leu Ile Arg Tyr
                                     570
                565
Gly Thr Ala Thr His Thr Val Asn Thr Asp Gln Arg Arg Ile Pro Leu
            580
                                585
Thr Leu Thr Asn Asn Gly Gly Asn Ser Tyr Ser Phe Gln Val Pro Ser
        595
                            600
                                                 605
Asp Ser Gly Val Ala Leu Pro Gly Tyr Trp Met Leu Phe Val Met Asn
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<211> 1917

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<220>

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D. Dendroides GaO	
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<212> DNA

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<212> DNA
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